REMARKS

Favorable reconsideration of the subject application is respectfully requested in view of the following remarks. By this amendment, claims 1 and 3-19 are canceled. This amendment is not to be construed as acquiescence to any rejection and is made without prejudice to prosecution of any subject matter modified by the amendment in a related divisional, continuation, or continuation-in-part application.

Approval of Petition to Withdraw Holding of Abandonment

Applicants thank the Examiner for noting that the Petition to Withdraw Holding of Abandonment, filed January 9, 2004, was approved.

Rejection Under 35 U.S.C. § 102(b)

Claims 21-25 stand rejected under 35 U.S.C. § 102(b), as allegedly anticipated by Brinkman *et al.* More specifically, the Examiner asserts that Brinkman *et al.* is referenced in NCBI entry NP_065144.1 (XAGE-1), which provides a polypeptide sequence of XAGE-1, having a 98% overall homology to SEQ ID NO:809. Based upon this reference, the Examiner appears to incorrectly draw the conclusion that Brinkman *et al.* discloses the polypeptide sequence provided in NCBI entry NP_065144.1 (XAGE-1).

Applicants traverse this basis of rejection and maintain that Brinkman *et al.* fails to describe the claimed invention, and, therefore, the Action has not established a *prima facie* case of anticipation.

As fully explained in the previous Amendment submitted August 21, 2003, Brinkman *et al.* absolutely fails to describe the claimed polypeptides, namely variants and fragments of SEQ ID NO:809. Contrary to the Examiner's assertion, Applicants submit that Brinkman *et al.* does not disclose the polypeptide sequence of XAGE-1. Indeed, Brinkman *et al.* explicitly state that they could not determine and, consequently, do not disclose the polypeptide sequence of XAGE-1. "Because of the uncertainty with translation, this gene was omitted from Fig. 3 [alignment of the deduced amino acid sequences of XAGE-2 and XAGE-3 with a typical GAGE gene]" (page 1447, column 2, lines 16-18). Thus, while Brinkman *et al.* describes the

identification of partial polynucleotide sequences (ESTs) corresponding to XAGE-1, it absolutely fails to identify or describe the XAGE-1 polypeptide sequence. Nor is the polypeptide sequence implicitly disclosed by Brinkman *et al.* based upon the identification of partial polynucleotide sequences, since the authors explicitly concede that the XAGE-1 polypeptide sequence could not be determined based upon the identified polynucleotide sequences. Since Brinkman *et al.* absolutely fails to provide the polypeptide sequence of XAGE-1, it clearly cannot anticipate the presently claimed polypeptide sequences.

Given that Brinkman *et al.* fails to provide the polypeptide sequence of XAGE-1, the Examiner has attempted to work backwards from Applicants' sequence (SEQ ID NO:809) to establish some basis on which to rely in his assertion that Brinkman *et al.* discloses the XAGE-1 polypeptide sequence and, therefore, anticipates the presently claimed invention. To this end, the Examiner searched the NCBI database to identify accession number NP_065144, which provides the XAGE-1 polypeptide sequence and lists Brinkman *et al.* in its list of related references. However, a review of the history of NCBI entry NP_065144 unequivocally states that "accession NP_065144.1 was first seen at NCBI on Sept 5 2000." Accordingly, this accession number was not publicly available until at least September 5, 2000, which is after the priority date afforded the instant application, which is at least as early as April 27, 2000, and, therefore, does not qualify as prior art under Section 102. For the Examiner's convenience, a copy of the Revision History for NP_065144, including this statement, is attached.

Furthermore, the fact that Brinkman *et al.* is provided in a list of publications related to the sequence listing in no way establishes that Brinkman *et al.* itself provides the listed sequence. In fact, as described in detail above, careful review of Brinkman *et al.* clearly reveals that Brinkman *et al.* fails to disclose the polypeptide sequence of XAGE-1 or the presently claimed sequences. In addition, Applicants emphasize that the comment provided directly below the listing of references in NCBI entry NP_065144.1 clearly states that the reference sequence disclosed therein was derived from AF251237.1, which is a full length cDNA sequence of XAGE-1. However, the AF251237.1 sequence was not seen by NCBI until August 3, 2000, according to the Revision History for AF251237.1, a copy of which is attached for the Examiner's convenience. This, of course, makes perfect sense, since the full length XAGE-1

cDNA and predicted open reading frame were not identified until the work of Liu *et al.*, described in Cancer Research 60: 4752-4755 (September 1, 2000)(copy attached), which was also published after the priority date of the instant application.

To summarize, Applicants submit that Brinkman *et al.* fails to anticipate the presently claimed invention, since Brinkman *et al.* fails to disclose the claimed sequences (and even explicitly admits that the authors were not in possession of the claimed polypeptides or sequences). In addition, Applicants submit that the XAGE-1 polypeptide sequence that the Examiner is attempting to cite against the present claims was not publicly available until at least September 5, 2000, according to NCBI records, which is after the priority date of the instant application. Accordingly, the instant claims are not anticipated by Brinkman *et al.* or the NCBI XAGE-1 sequence. Applicants respectfully request that the Examiner reconsider and withdraw this basis of rejection.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Applicants submit that all of the claims remaining in the application are clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

Carol D. Laherty, Ph.D.
Registration No. 51,909

CDL:jto
Enclosures:
Postcard
Copy of Revision History for NP_065144
Copy of Revision History for AF251237.1
Copy of Liu *et al.*, Cancer Research 60, 4752-4755

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486518_1.DOC

Express Mail No. EV449559520US

NCBI

Sequence Revision History

Revision history for NP_065144

GI	Version	Update Date	Status	I	II
9966899	1	Dec 21 2003 9:43 AM	Live	◉	○
9966899	1	Oct 5 2003 12:40 AM	Dead	○	◉
9966899	1	Sep 6 2003 6:54 PM	Dead	○	○
9966899	1	Apr 6 2003 9:56 AM	Dead	○	○
9966899	1	Mar 27 2002 3:10 PM	Dead	○	○
9966899	1	Feb 11 2002 7:17 AM	Dead	○	○
9966899	1	Dec 19 2001 10:31 AM	Dead	○	○
9966899	1	Apr 24 2001 12:47 PM	Dead	○	○
9966899	1	Nov 2 2000 11:34 AM	Dead	○	○
9966899	1	Sep 5 2000 8:43 AM	Dead	○	○

Accession NP_065144.1 was first seen at NCBI on Sep 5 2000 8:43 AM

Express Mail No. EV449559520US

NCBI

Sequence Revision History

Revision history for AF251237.1

GI	Version	Update Date	Status
9885324	1	Aug 23 2000 12:04 AM	Live

Accession AF251237 was first seen at NCBI on Aug 23 2000 12:04 AM

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[CANCER RESEARCH 60, 4752–4755, September 1, 2000]

Advances in Brief

XAGE-1, A New Gene That Is Frequently Expressed in Ewing's Sarcoma[1]

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Abstract

Our previous expressed sequence tag database analysis indicates that *XAGE-1* is frequently found in Ewing's sarcoma and alveolar rhabdomyosarcoma (U. Brinkmann *et al.*, Cancer Res., *59:* 1445–1448, 1999). Using Northern blots and RNA dot blots, we have now found that *XAGE-1* is highly expressed in normal testis, in seven of eight Ewing's cell lines, in four of nine Ewing's sarcoma patient samples, and in one of one alveolar rhabdomyosarcoma patient sample. The gene is located on the X chromosome. The full-length cDNA contains 611 bp and predicts a protein of M_r 16,300 with a potential transmembrane domain at the NH_2 terminus. *XAGE-1* shares homology with GAGE/PAGE proteins in the COOH-terminal end. These findings could be valuable for cancer diagnosis and cancer immunotherapy.

Introduction

Large numbers of ESTs[3] have been cloned from various tissues and cancers (1, 2). Each cDNA clone or EST sequence is generated from a single transcript. The frequency and distribution of the many different transcripts in a given tissue depends on the level of gene expression. Therefore, a particular gene expression pattern can be frequently predicted by analysis of the frequency and specificity of various EST sequences. We previously reported a computer screening strategy that identified genes that are preferentially expressed in prostate or prostate tumors (3–5). From this screen, numerous genes were identified including a novel gene, *PAGE4* (previously named *PAGE1*) (6), and a set of *XAGE* genes (7), which are related to the GAGE/MAGE family of melanoma associated CT antigens.

CT antigens are a distinct class of differentiation antigens that have a restricted pattern of expression in normal tissues (8–10). CT antigens are currently viewed as attractive candidates for cancer vaccines. Some thoroughly studied CT antigens are MAGE, BAGE, GAGE, and LAGE/NY-ESO-1 (9–17). These genes are primarily expressed in the primitive germ cells, spermatogonia, in the normal testis. Malignant transformation is often associated with activation or derepression of silent CT genes, and this results in the expression of CT antigens in a variable proportion of a wide range of human tumors. Recently, several additional members were added to the CT antigen family. These include various PAGEs, PRAME, SSX, SCP-1, CT7, MAGEC1, and MAGED1 (6, 11, 18–22). Identification of new CT antigens or new family members is still being actively pursued in the cancer research field.

Previously, we identified three related genes that we termed *XAGE*s by homology walking using the dbEST database (7). ESTs of the *XAGE* group were found in cDNA libraries from testis, Ewing's sarcoma, alveolar rhabdomyosarcoma, fetal tissues, and germ cell tumors by database analysis. In this study, we describe the isolation of a full-length cDNA of *XAGE-1* and analysis of the expression of the *XAGE-1* gene. We found that the gene is highly expressed in normal testis, with aberrant expression in sarcomas of various types. Although XAGE-1 did not show overall homology with any polypeptide recorded in the data bank, it has strong homology with members of GAGE/PAGE family in the COOH terminus of the predicted protein, indicating that XAGE-1 belongs to a new family of CT antigens.

Materials and Methods

Tissues and Cell Lines. Ewing's tumor tissue was obtained from frozen specimens obtained from patients treated at the National Cancer Institute. Rhabdomyosarcoma tumor tissue was obtained from the Cooperative Human Tissue Network, Children's Cancer Group. All alveolar rhabdomyosarcoma tumor specimens were found to express the PAX-3-FKHR fusion transcript by RT-PCR.[4] Osteosarcoma cell lines were obtained from the American Type Culture Collection. Ewing's sarcoma cell lines RD-ES, TC-32, TC-71, and 5838 have been described previously, and all contain EWS-FLI-1 fusion transcripts (23).[4] LD, LG, JM, and SB are cell lines established in our laboratories (by L. J. H.) directly from tumor specimens. The cell line JM does not express an EWS-ETS fusion transcript.

Northern Blots and RNA Dot Blot. RNA was extracted either from tumor tissue using Trizol (Life Technologies, Inc.) or from cell lines using RNAeasy (Qiagen). Total RNA (20 μg) was used for Northern blot analysis of sarcoma tumors. The multiple tissue mRNA dot blot and the normal tissue Northern blot were purchased from Clontech (Palo Alto, CA). The 450-bp probe used for hybridization was generated from EST clone af89d01.s1 by digestion with *Eco*RI and *Not*I. The hybridization was conducted as follows: the RNA-containing membranes were blocked for 3 h at 45°C in hybridization solution. Probes labeled with ^{32}P either by random primer extension or by end labeling (Lofstrand Labs Limited) were added to the membrane and hybridized for 15 h at 45°C. Membranes were washed twice with 2× SSC/0.1% SDS at room temperature and twice with 0.1× SSC/0.1% SDS at 65°C. The membranes were exposed to X-ray film for 1–2 days before development

The Southern blot of human chromosomes (Oncor, Gaithersburg, MD) was conducted using the same probe and the same hybridization conditions used for Northern blot.

RT-PCR was performed on cDNA from 24 different human tissues using human rapid-scan gene expression panels (Origene, Inc., Rockville, MD). The thermocycling protocol was initial denaturation at 94°C for 3 min and 35 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and elongation at 72°C for 3 min. The PCR reactions were analyzed on agarose gels, and specific products were cloned into TA vectors (Invitrogen) and sequenced on an automated capillary sequencer using Perkin-Elmer's dRhodamine terminator cycle sequencing kit (Perkin-Elmer Applied System).

The primers used were as follows: (*a*) xa-1, 5′-CAGCTTGTCTTCATTTAAACTTGTGGTTGC-3′; (*b*) xa-2, 5′-TCCCAGGAGCCCAGTAATGGAGA-3′; (*c*) xa-8, 5′-ACCTGGGAAGGAGCATAGGA-3′; and (*d*) xa-10, 5′-CTTTATTGAGATAGTTTAAGTCAAATATCTAA-3′. The oligonucleotides were synthesized by Sigma-Genosys.

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[1] Supported in part by the Children's Cancer Group/Cooperative Human Tissue Network, which is funded by the National Cancer Institute.

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[3] The abbreviations used are: EST, expressed sequence tag; CT, cancer-testis; RT-PCR, reverse transcription-PCR; PBL, peripheral blood leukocyte.

[4] Unpublished observations.

Results

Restricted Expression in Normal Tissues. To determine the relative expression of *XAGE-1* mRNA in different tissues and tumors, we first conducted a mRNA dot blot (Clontech) analysis using a full insert of EST af89d01.s1 as a labeling probe. Among the 61 different samples of normal tissues and 7 fetal tissues including lung, brain, liver, heart, and spleen, the expression of *XAGE-1* was detected only in testis (Fig. 1*A*). This result indicates that *XAGE-1*, like other CT antigens, is present in testis.

To verify the specificity of *XAGE-1* expression, we conducted a RT-PCR analysis by using the human rapid-scan panel with primers xa-1 and xa-2. We detected a 275-bp fragment in testis among the 24 different tissues analyzed (Fig. 1*B*). Unexpectedly, the 275-bp fragment was also present at lower amounts in normal lung and PBLs. Extremely weak expression of *XAGE-1* was detected in bone marrow, spleen, and skin. To compare the relative level of *XAGE-1* in testis, lung, and PBLs, different dilutions of cDNA were analyzed in the same rapid-scan panel as shown in Fig. 1*C*. The mRNA present in testis was about 10–100 times higher than that in the lung and more than 100 times higher than that in PBLs.

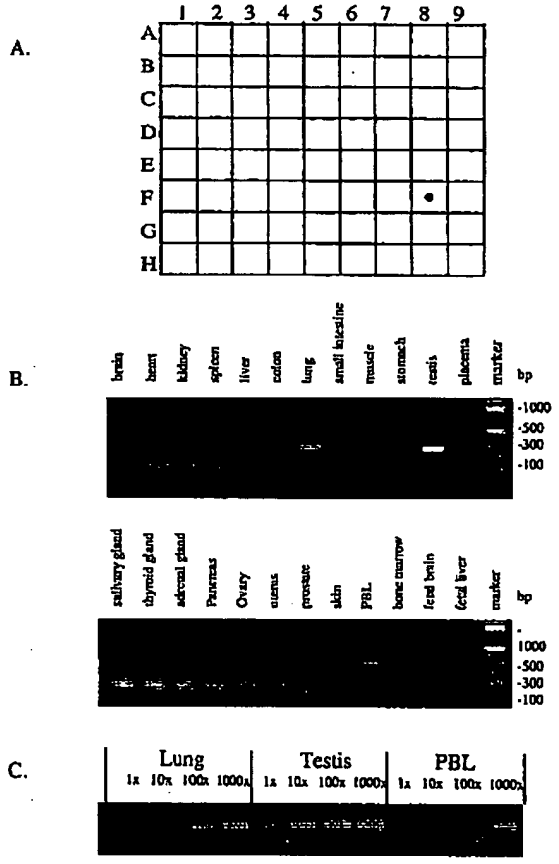

Fig. 1. Specific expression of *XAGE-1*. *A*, RNA dot blot hybridization. Human multiple tissue master blot with 76 tissue-specific poly(A) RNA (Clontech) were hybridized with XAGE-specific probe. *A1*, whole brain; *B1*, cerebral cortex; *C1*, frontal lobe; *D1*, parietal lobe; *E1*, occipital lobe; *F1*, temporal lobe; *G1*, p.g. of cerebral cortex; *H1*, pons; *A2*, cerebellum left; *B2*, cerebellum right; *C2*, corpus callosum; *D2*, amygdala; *E2*, caudate nucleus; *F2*, hippocampus; *G2*, medulla oblongata; *H2*, putamen; *A3*, substantia; *B3*, nucleus accumbens; *C3*, thalamus; *D3*, pituitary gland; *E3*, spinal cord; *A4*, heart; *B4*, aorta; *C4*, atrium left; *D4*, atrium right; *E4*, ventricle left, *F4*, ventricle right; *G4*, interventricular septum; *H4*, apex of the heart; *A5*, esophagus; *B5*, stomach; *C5*, duodenum; *D5*, jejunum; *E5*, ileum; *F5*, ileocecum; *G5*, appendix; *H5*, colon ascending; *A6*, colon transverse; *B6*, colon descending; *C6*, colon rectum; *A7*, kidney; *B7*, skeletal muscle; *C7*, spleen; *D7*, thymus; *E7*, PBLs; *F7*, lymph node; *G7*, bone marrow; *H7*, trachea; *A8*, lung; *B8*, placenta; *C8*, bladder; *D8*, uterus; *E8*, prostate; *F8*, testis; *G8*, ovary; *A9*, liver; *B9*, pancreas; *C9*, adrenal gland; *D9*, thyroid, gland; *E9*, salivary gland; *F9*, mammary gland. *B*, RT-PCR analysis. Twenty-four different sources of cDNA (Origene) were amplified by using primers xa-1 and xa-2 under the conditions described in "Materials and Methods." After the PCR cycles, 5 μl of 25 μl of the high-concentration (1000×) reactions were analyzed in agarose gel and visualized by staining with ethidium bromide. *C*, four different concentrations from lung, testis, and PBLs amplified in *A* were run together to compare the relative level of *XAGE-1*expression. *F3–H3, D6–H6, H8, G9,* and *H9* are empty.

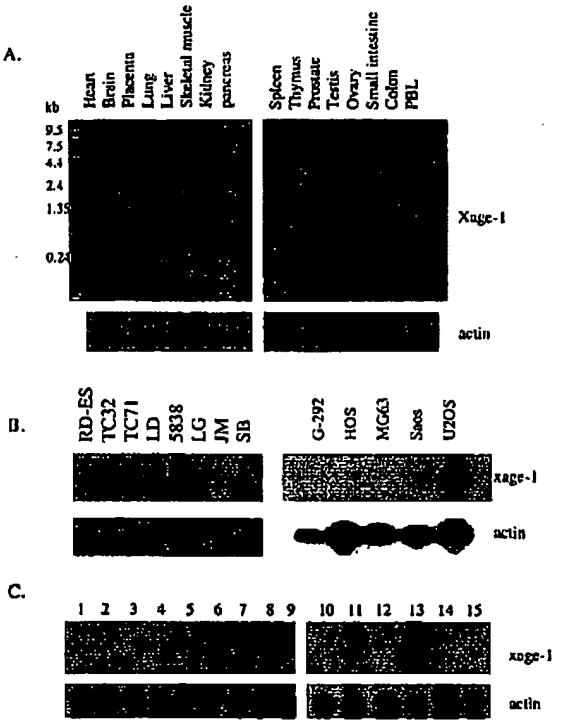

Fig. 2. Northern blot analysis of the *XAGE-1* gene. *A*, Northern blot (Clontech) of 16 different normal tissues probed separately with xage-1 and actin probe. *B*, the expression of *XAGE-1* in Ewing's sarcoma cell lines (*left panel*) and osteosarcoma cell lines (*right panel*). *C*, the expression of *XAGE-1* in patient tumor samples. *Lanes 1–9*, Ewing's sarcoma patients; *Lane 10*, normal muscle; *Lane 11*, alveolar rhabdomyosarcoma; *Lane 12*, fetal muscle; *Lanes 13–15*, embryonal rhabdomyosarcoma. Total RNA was probed separately with xage-1 probe and actin probe.

Because *XAGE-1* is highly abundant in testis and is expressed at a low level in lung and PBLs, we attempted to determine the transcript size in these different tissues. Northern blot analysis was conducted using the same probe used for the RNA dot blot. As shown in Fig. 2*A*, a single band of 700 bp was revealed in the testis. However, no signal was detected in lung and PBLs. This result is probably due to the low level of *XAGE-1* expression in lung and PBLs because the Northern blot analysis is much less sensitive than RT-PCR in detecting the expression of *XAGE-1*. These results are consistent with the RNA dot blot analysis described above (Fig. 1*A*).

***XAGE-1* Expression in Ewing's Sarcoma, Rhabdomyosarcoma, and Osteosarcoma.** Analysis of the EST database predicts that *XAGE-1* is present in Ewing's sarcoma and alveolar rhabdomyosarcoma. To confirm the database prediction experimentally, we first determined whether *XAGE-1* was present in the various Ewing's cell lines by Northern blot analysis. A single band of 700 bp was detected in seven of eight cell lines (Fig. 2*B*). *XAGE-1* was not expressed in cell line JM, which is a mouse xenograft tumor derived from a Ewing's sarcoma that has lost the chromosome translocation t(11;22).[4] Cell line 5838 had an extra band with a size of 1.2 kb. This band might be due to alternate splicing or use of an alternate polyadenalytion signal in the *XAGE-1* gene.

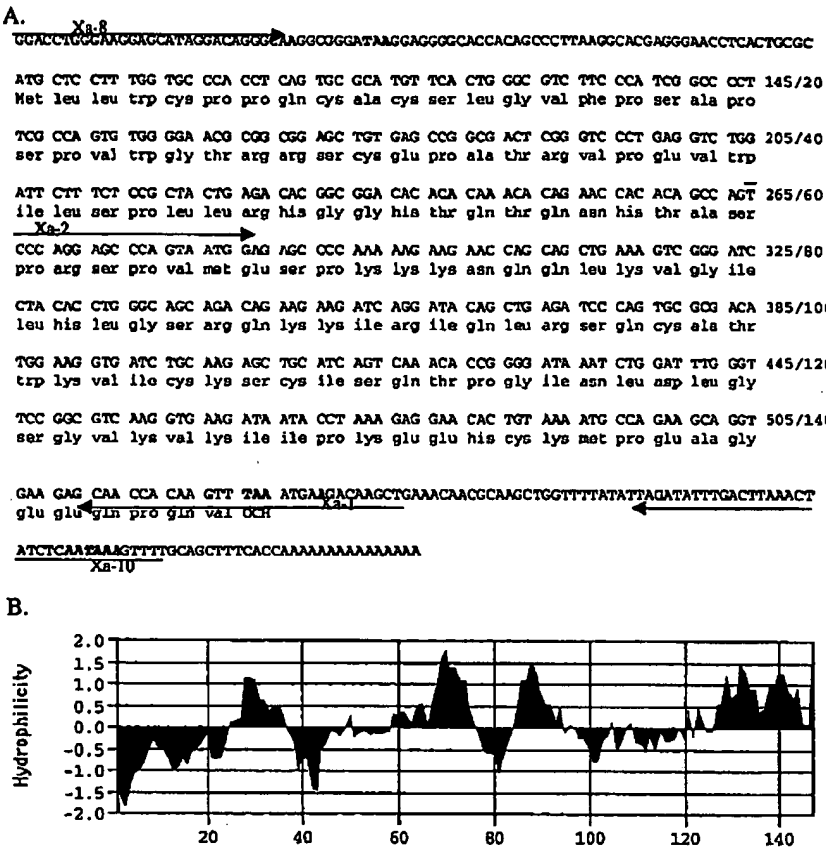

Fig. 3. Sequence of *XAGE-1*. *A*, the nucleotide and amino acid sequence of XAGE-1. The polyadenylation signal is *italicized* and *bold*. The translational stop codons are indicated in *bold*. Primers are showed by *arrows*. *B*, Hoop/Woods hydrophilicity plot. A potential membrane-spanning region was noticed in the NH_2-terminal end.

XAGE-1 was present in two of five osteosarcoma cell lines, with the SAOS cell line showing relatively low *XAGE-1* expression (Fig. 2*B*).

To address whether *XAGE-1* was present in human patient samples, we conducted a Northern blot hybridization analysis. Of nine patients with Ewing's sarcoma, four (patients 5–8) expressed *XAGE-1* with a single 700-bp band (Fig. 2*C*). Patients 1 and 5–9 expressed the *EWS-FLI-1* transcript, an indication of chromosome translocation (Ref. 24; data not shown). We noticed that *XAGE-1* was not expressed in all of the patient samples with the chromosome translocation. However, samples that did not express either an *EWS-FLI-1* or an *EWS-ERG* fusion transcript also did not express *XAGE-1* (Fig. 2*C*, *Lanes 2–4*). The correlation between the absence of *XAGE-1* expression and the absence of chromosome translocation needs further study. *XAGE-1* was also expressed in one of one patient sample with alveolar rhabdomyosarcoma and in one of three patient samples of embryonal rhabdomyosarcoma but not in the normal controls (Fig. 2*C*). Together, these data indicate that *XAGE-1* is expressed in nearly half of the sarcoma patient samples.

Chromosome Localization of *XAGE-1*. Most of the CT antigens are localized on the X chromosome, with the exception of SCP-1, which is located on chromosome 1 (8, 20). To find where *XAGE-1* is localized, Southern blot hybridization was performed on a human chromosome blot using the same probe used for dot blot and Northern blot. We detected only one strong band on the X chromosome, and no other cross-hybridizing bands were found on the blot (data not shown). This result indicates that the *XAGE-1* gene is located on the X chromosome and that there is not a very strong homology with the other predicted *XAGE* members, *XAGE-2* and *XAGE-3*, because under stringent hybridization conditions, *XAGE-2* and *XAGE-3* were not detected.

Full-length cDNA of *XAGE-1* and Putative Peptide Sequences. To obtain the full-length *XAGE-1* cDNA sequence, rapid amplification of cDNA ends-PCR was performed using primers localized in the

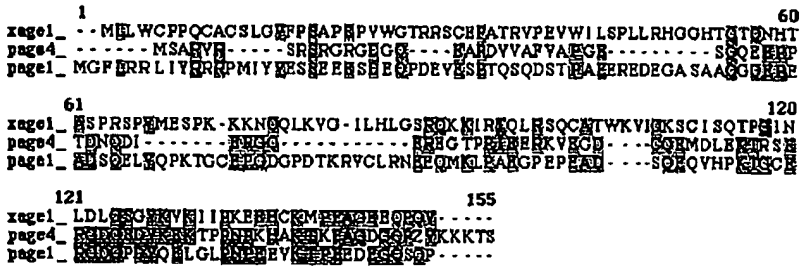

Fig. 4. Comparison of the XAGE-1, PAGE-4, and PAGE-1 (GAGE9) amino acid sequences. Conserved residues are *shaded*.

EST contig and total RNA from Ewing's sarcoma cell line TC-71. The longest rapid amplification of cDNA ends product contains an additional 184 nucleotides at the 5′ end compared with the EST contig sequence. The correct cDNA sequence was confirmed by sequencing the PCR product from primers xa-8 and xa-10. The *XAGE-1* cDNA is 611 bp in length, excluding the poly(A) tail, and contains 438 nucleotides in the coding region, flanked by 85 bp in the 5′-untranslated region and 88 bp in the 3′-untranslated region (Fig. 3*A*).

The longest open reading frame indicates that the putative XAGE-1 protein consists of 146 amino acid residues with a molecular weight of 16,300. Hydrophilicity analysis (Fig. 3*B*) indicates a hydrophobic sequence in the NH_2-terminal end, suggesting the possibility of a transmembrane domain. Analysis of the protein sequence reveals no possible posttranslational modifications by searching GCG Lite. This putative protein did not show overall sequence homology with any peptide recorded in the data banks. However, alignment of the amino acid sequence of XAGE-1 with PAGE4 (6) and PAGE1 [Ref. 25 (renamed GAGE9; Ref. 15)] reveals a striking homology in the COOH-terminal end of these proteins (Fig. 4), suggesting that *XAGE-1* encodes a distinct protein that could share structural or functional features with other GAGE/PAGE family members.

Discussion

XAGE-1 is a human X-linked gene that is strongly expressed in normal testis, Ewing's sarcoma, and alveolar rhabdomyosarcoma. It is also expressed in normal lung and PBLs, but at much lower levels. *XAGE-1* expression in lung and lymphocytes was not predicted by EST database analysis, probably because of its weak expression and the small size of lung and PBL libraries. The expression pattern of *XAGE-1* in Ewing's sarcoma, lung, PBLs, and bone marrow is interesting. At present, it is unclear in which cell type Ewing's sarcoma originates and why it metastasizes to the lung. The high level of *XAGE-1* expression in many Ewing's sarcomas suggests that the tumor could arise in cells in the bone or bone marrow that normally express *XAGE-1* or metastasize to the region where *XAGE-1* is expressed. Additional experiments using *in situ* hybridization or immunohistochemistry should provide information about this possible relationship.

Until now, there has been no report on the expression of CT antigens in Ewing's sarcoma and alveolar rhabdomyosarcoma. We found *XAGE-1* expressed in seven of eight Ewing's sarcoma cell lines, 44% (four of nine) of Ewing's sarcoma tumor specimens, and one of one alveolar rhabdomyosarcoma tumor sample. We are currently screening the expression of *XAGE-1* in other tumor cell types. Our preliminary data indicate that the *XAGE-1* gene is activated in many tumor cell lines and other tumor types. Studies on the *XAGE-1* gene may provide more information on this cancer antigen family that may be of use in the diagnosis or immunotherapy of cancer.

Acknowledgments

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References

1. Adams, M. D., Kerlavage, A. R., Fleischmann, R. D., Fuldner, R. A., Bult, C. J., Lee, N. H., Kirkness, E. F., Weinstock, K. G., Gocayne, J. D., White, O., Sutton, G., Blake, J. A., Brandon, R. G., Chiu, M. W., Clayton, R. A., Cline, R. T., Cotton, M. D., Earlehughes, J., Fine, L. D., Fitzgerald, L. M., Fitzhugh, W. M., Fritchman, J. L., Geoghagen, N. S. M., Glodek, A., Gnehm, C. L., Hanna, M. C., Hedblom, B., Hinkle, P. S., Kelley, J. M., Klimek, K. M., Kelley, J. C., Liu, L. I., Marmaros, S. M., Merrick, J. M., Morenopalanques, R. F., McDonald, I. A., Nguyen, D. T., Pellegrino, S. M., Phillips, C. A., Ryder, S. E., Scott, J. L., Saudek, D. M., Shirley, R., Small, K. V., Spriggs, T. A., Utterback, T. R., Weldman, J. F., Li, Y., Barthlow, R., Bednarik, D. P., Cao, L. A., Cepeda, M. A., Coleman, T. A., Collins, E. J., Dimke, D., Feng, P., Ferrie, A., Fischer, C., Hastings, G. A., He, W. W., Hu, J. S., Huddleston, K. A., Greene, J. M., Gruber, J., Hudson, P., Kim, A., Kozak, D. L., Kunsch, C. Ji, H. J., Li, H. D., Meissner, P. S., Olsen, H., Raymond, L., Wei, Y. F., Wing, J., Xu, C., Yu, G. L., Ruben, S. M., Dillon, P. J., Fannon, M. R. Rosen, C. A., Haseltine, W. A., Fields, C., Fraser, C. M., and Venter, J. C. Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. Nature (Lond.), *377:* 3–174, 1995.
2. Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merril, C. R., Wu, A., Olde, B., Moreno, R. F., Kerlavage, A. R., McCombie, W. R., and Venter, J. C. Complementary DNA sequencing: expressed sequence tags and human genome project. Science (Washington DC), *252:* 1651–1656, 1991.
3. Liu, X., Essand, M., Vasmatzis, G., Lee, B., and Pastan, I. Identification of three new alternate human kallikrein 2 transcripts: evidence of long transcript and alternative splicing. Biochem. Biophys. Res. Commun., *264:* 833–839, 1999.
4. Vasmatzis, G., Essand, M., Brinkmann, U., Lee, B., and Pastan, I. Discovery of three genes specifically expressed in human prostate by expressed sequence tag database analysis. Proc. Natl. Acad. Sci. USA, *95:* 300–304, 1998.
5. Essand, M., Vasmatzis, G., Brinkmann, U., Duray, P., Lee, B., and Pastan, I. High expression of a specific T-cell receptor γ transcript in epithelial cells of the prostate. Proc. Natl. Acad. Sci. USA, *96:* 9287–9292, 1999.
6. Brinkmann, U., Vasmatzis, G., Lee, B., Yerushalmi, N., Essand, M., and Pastan, I. *PAGE-1*, an X chromosome-linked GAGE-like gene that is expressed in normal and neoplastic prostate, testis, and uterus. Proc. Natl. Acad. Sci. USA, *95:* 10757–10762, 1998.
7. Brinkmann, U., Vasmatzis, G., Lee, B., and Pastan, I. Novel genes in the PAGE and GAGE family of tumor antigens found by homology walking in the dbEST database. Cancer Res., *59:* 1445–1448, 1999.
8. De Smet, C., Lurquin, C., Plaen, E. D., Brasseur, F., Zarour, H., De Backer, O., Coulie, P. G., and Boon, T. Genes coding for melanoma antigens recognised by cytolytic lymphocytes. Eye, *11:* 243–248, 1997.
9. Chen, Y. T., and Old, L. J. Cancer-testis antigens: targets for cancer immunotherapy. Cancer J. Sci. Am., *5:* 16–17, 1999.
10. Gillespie, A. M., Rodgers, S., Wilson, A. P., Tidy, J., Rees, R. C., Coleman, R. E., and Murray, A. K. MAGE, BAGE, and GAGE. tumour antigen expression in benign and malignant ovarian tissue. Br. J. Cancer, *78:* 816–821, 1998.
11. Lucas, S., De Smet, C., Arden, K. C., Viars, C. S., Lethe, B., Lurquin, C., and Boon, T. Identification of a new *MAGE* gene with tumor-specific expression by representational difference analysis. Cancer Res., *58:* 743–752, 1998.
12. Jungbluth, A. A., Busam, K. J., Kolb, D., Iversen, K., Coplan, K., Chen, Y. T., Spagnoli, G. C., and Old, L. J. Expression of MAGE-antigens in normal tissues and cancer. Int. J. Cancer, *85:* 460–465, 2000.
13. Chen, Y. T., Gure, A. O., Tsang, S., Stockert, E., Jager, E., Knuth, A., and Old, L. J. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc. Natl. Acad. Sci. USA, *95:* 6919–6923, 1998.
14. Boel, P., Wildmann, C., Sensi, M. L., Brasseur, R., Renauld, J. C., Coulie, P., Boon, T., and van der Bruggen, P. *BAGE*: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity, *2:* 167–175, 1995.
15. Backer, O., Ardon, K. C., Boretti, M., Vantomme, V., Smet, C. D., Czekay, C. S., Plean, V., Brasseur, F., Chomezq, P., Eynde, B. V., Boon, T., and Bruggen, P. Characterization of the *GAGE* genes that are expressed in various human cancers and in normal testis. Cancer Res., *59:* 3157–3165, 1999.
16. De Plaen, E., Arden, K., Traversari, C., Gaforio, J. J., Szikora, J. P., Desmet, C., Brasseur, F., van der Bruggen, P., Lethe, B., Lurquin, C., Brasseur, R., Chomez, P., Debacker, O., Cavenee, W., and Boon, T. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. Immunogenetics, *40:* 360–369, 1994.
17. Chen, Y. T., Boyer, A. D., Viars, C. S., Tsang, S., Old, L. J., and Arden, K. C. Genomic cloning and localization of *CTAG*, a gene encoding an autoimmunogenic cancer-testis antigen NY-ESO-1, to human chromosome Xq28. Cytogenet. Cell Genet., *79:* 237–240, 1997.
18. Gure, A. O., Stockert, E., Arden, K. C., Boyer, A. D., Viars, C. S., Scanlan, M. J., Old, L. J., and Chen, Y. T. CT10: a new cancer-testis (CT) antigen homologous to CT7 and the MAGE family, identified by representational-difference analysis. Int. J. Cancer, *85:* 726–732, 2000.
19. Tureci, O., Chen, Y. T., Sahin, U., Gure, A. O., Zwick, C., Villena, C., Tsang, S., Seitz, G., Old, L. J., and Pfreundschuh, M. Expression of *SSX* genes in human tumors. Int. J. Cancer, *77:* 19–23, 1998.
20. Tureci, O., Sahin, U., Zwick, C., Koslowski, M., Seitz, G., and Pfreundschuh, M. Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. Proc. Natl. Acad. Sci. USA, *95:* 5211–5216, 1998.
21. Pold, M., Zhou, J., Chen, G. L., Hall, J. M., Vescio, R. A., and Berenson, J. R. Identification of a new, unorthodox member of the *MAGE* gene family. Genomics, *59:* 161–167, 1999.
22. Watari, K., Tojo, A., Nagamura-Inoue, T., Nagamura, F., Takeshita, A., Fukushima, T., Motoji, T., Tani, K., and Asano, S. Identification of a melanoma antigen, PRAME, as a BCR/ABL-inducible gene. FEBS Lett., *466:* 367–371, 2000.
23. Van Valen, F. Ewing's Sarcoma Family of Tumors in Human Cell Culture, Vol. 1. Boston, MA: Kluwer Academic Publishers, 1999.
24. Sorensen, P. H., Lessnick, S. L., Lopez-Terrada, D., and Al, A. A second Ewing's sarcoma translocation, t(21;22), fuses the *EWS* gene to another ETS-family transcription factor, ERG. Nat. Genet., *6:* 146–151, 1994.
25. Chen, M. E., Lin, S-H., Chung, L. W. K., and Sikes, R. A. Isolation and characterization of PAGE-1 and GAGE-7. J. Biol. Chem., *273:* 17618–17625, 1998.